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Review

Traction force microscopy on soft elastic substrates: A guide to recent computational advances☆

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ABSTRACT

The measurement of cellular traction forces on soft elastic substrates has become a standard tool for many labs working on mechanobiology. Here we review the basic principles and different variants of this approach. In general, the extraction of the substrate displacement field from image data and the reconstruction procedure for the forces are closely linked to each other and limited by the presence of experimental noise. We discuss different strategies to reconstruct cellular forces as they follow from the foundations of elasticity theory, including two- versus three-dimensional, inverse versus direct and linear versus non-linear approaches. We also discuss how biophysical models can improve force reconstruction and comment on practical issues like substrate preparation, image processing and the availability of software for traction force microscopy. This article is part of a Special Issue entitled: Mechanobiology.

1. Introduction

Over the last two decades, it has become apparent that mechanical forces play a central role for cellular decision-making, leading to the emerging field of mechanobiology [1,2]. In order to understand how forces impact cellular processes, it is essential to measure them with high spatiotemporal resolution and to correlate them either statistically or causally with the cellular process of interest. The most common approach is to measure forces at the cell–matrix interface. This field has grown rapidly over the last years and has become to be known as *traction force microscopy* (TFM). Using this approach, it has been shown e.g. that cellular traction often correlates with the size of adhesion contacts [3–7] but also that this correlation depends on the growth history of the adhesion contact under consideration [8,9]. For most tissue cell types, high extracellular stiffness correlates with large traction forces and large cell–matrix adhesion contacts. These large contacts are thought to not only ensure higher mechanical stability, but also to reflect increased signaling activity. This leads to a stiffness-sensitive response of cells, e.g. during cell spreading and migration [10,11] or stem cell differentiation [12–15]. While TFM has become a standard tool in many labs working on mechanobiology, in practice the details of its implementation vary significantly and the development of new approaches is moving forward at a very fast pace.

From a general point of view, forces are not an experimentally directly accessible quantity but have to be infered from the fact that they create some kind of motion. Despite the fact that this motion can follow different laws depending on the details of the system under consideration (e.g. being elastic or viscous), a force measurement essentially requires to monitor some kind of dynamics. This is illustrated best with a linear elastic spring. Here force is defined as $F = kx$, with spring constant k and displacement x. Without a measurement of x, no statement on F would be possible (k is a constant that can be obtained from a calibration experiment). In order to measure x, the reference state $x = 0$ has to be known, and therefore one typically needs a relaxation process to determine the absolute value of x. Thus even seemingly static situations require some dynamical measurement. Another instructive example is the stress acting over a fictitious surface inside a static but strained elastic body. In order to measure this stress directly, in principle one has to cut the surface open and to introduce a strain gauge that measures forces by the movement of a calibrated spring. Alternatively one needs to use a model that allows one to predict this stress from an elastic calculation.

In summary, each direct measurement of cellular forces has to start with the identification of a suitable strain gauge. Thus a helpful classification of the wide field of TFM can be introduced by considering the different ways in which a strain gauge can be incorporated in a cell culture setup (Fig. 1). The most obvious way to do this is to replace the glass or plastic dishes of cell culture by an elastic system that can deform under cell forces. Early attempts to do so used thin elastic sheets, which buckle under cellular traction and thus provide an immediate visual readout

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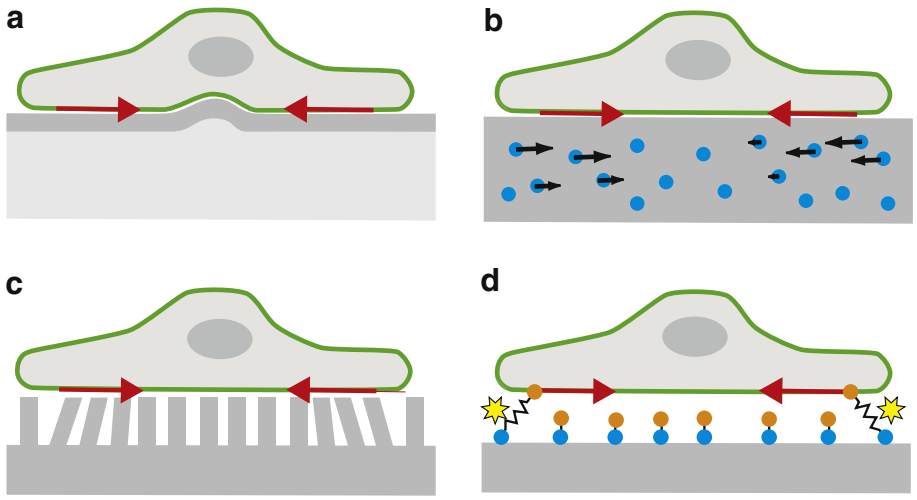

Fig. 1. Schematic representation of different setups for traction force microscopy. (a) Thin films buckle under cell traction, therefore this setup is difficult to evaluate quantitatively. (b) The standard setup are thick films with embedded marker beads as reviewed here. The substrate deformation field can be extracted with image processing and has to be deconvoluted to obtain the cellular traction field. (c) Pillar arrays are local strain gauges and do not require any deconvolution; however, they also present topographical and biochemical patterns to cells. (d) Fluorescent stress sensors typically use the relative movement of two molecular domains connected by a calibrated elastic linker to create a fluorescent signal, e.g. by Förster resonance energy transfer (FRET) or by quenching.

(Fig. 1 a) [16]. However, due to this non-linear response, it is difficult to evaluate these experiments quantitatively. Therefore this assay was first improved by using thin silicone films under tension [17] and then thick polyacrylamide (PAA) films that do not buckle but deform smoothly under cell traction (Fig. 1 b) [18]. Today the use of thick films made of different materials is a standard approach in many mechanobiology labs. Fiducial markers can be embedded into these substrates and their movement can be recorded to extract a displacement field. Solving the inverse problem of elasticity theory, cellular traction forces can be calculated from these data [18–22]. An interesting alternative to solving the inverse problem is the direct method that constructs the stress tensor by a direct mapping from a strain tensor calculated from the image data [23–25]. Here we will review these methods that are based on the experimental setup shown in Fig. 1 b.

A simple alternative to TFM on soft elastic substrates is the use of pillar arrays, where forces are decoupled in an array of local strain gauges (Fig. 1 c) [4,7,26–28]. Pillars can be microfabricated from many different materials, including elastomeres like polydimethylsiloxane (PDMS) or solid material like silicium, as long as they have a sufficiently high aspect ratio to deform under cellular traction. One disadvantage of this approach is that cells are presented with topographical cues and that their adhesion sites grow on laterally restricted islands, making this system fundamentally different from unconstrained adhesion on flat substrates. Moreover it has recently been pointed out that substrate warping might occur if the base is made from the same elastic material, thus care has to be taken to correctly calibrate these systems [29].

A very promising alternative to macroscopically large elastic strain gauges is the use of molecular force sensors (Fig. 1 d) [30–36]. Such a sensor typically consists of two molecular domains connected by a calibrated elastic linker. In the example for an extracellular sensor shown in Fig. 1 d, the distal domain is bound to a gold dot on the substrate that quenches the cell-bound domain and fluorescence ensues as the linker is stretched by cellular forces [35]. For intracellular sensors, one can use Förster resonance energy transfer (FRET), which means that fluorescence decreases as the linker is stretched [30]. Fluorescent stress sensors give a direct readout of molecular forces, but for several reasons one has to be careful when interpreting these signals. First the effective spring constant of the elastic linker might depend on the local environment in the cell, even if calibrated in a single-molecule force spectroscopy experiment. Second the fluorescent signal is a sensitive function of domain separation and relative orientation, thus a direct conversion into force can be problematic. Third it is difficult to control the number of engaged sensors, thus the fluorescent signal cannot easily be integrated over a larger region. Fourth the molecular stress sensor reads out only part of the force at work in the cellular structure of interest (e.g. the adhesion contact). Therefore fluorescent stress sensors are expected to complement but not to replace traditional TFM in the future.

One advantage of fluorescent stress sensors over soft elastic substrates and pillar assays is that they can be more easily adapted to force measurements in tissue, for example in developmental systems with fast and complicated cell rearrangements, although the same issues might apply as discussed above for single cells. Recently, however, it has been shown that macroscopic oil droplets can be used to monitor forces during developmental processes [37]. In principle, also subcellular structures such as focal adhesions, stress fibers, mitochondria or nuclei can be used as fiducial markers for cell and tissue deformations [38,39]. One disadvantage of this approach however is that subcellular structures are usually highly dynamic and can exhibit their own modes of movement, thus not necessarily following the overall deformation of the cell. Nevertheless conceptually and methodologically these approaches are similar to traditional TFM and also work in the tissue context.

Another important subfield of TFM is estimating internal forces from cell traction using the concept of force balance. This concept has been implemented both for forces between few cells [40,41] and for forces within laterally extended cell monolayers [42,43]. In the latter case (*monolayer stress microscopy*), one assumes that the cell monolayer behaves like a thin elastic film coupled to the underlying matrix by stresses (alternatively one can assume coupling by strain [44,45]). Combined with a negative pressure that represents the effect of actomyosin contractility, the physics of thin elastic films is now increasingly used to describe forces of cell monolayers in general [46–49]. Recently single cell and monolayer approaches for internal force reconstruction have been combined by tracking each cell inside a monolayer [50]. For single cells, the combination of modeling and TFM has recently been advanced to estimate the tensions in the whole set of stress fibers within cells on pillar arrays [51] and soft elastic substrates [52]. For the latter case an actively contracting cable network constructed from image data has been employed to model contractility in the set of stress fibers within U2OS cells.

Despite the many exciting developments in the large field of TFM, the most commonly used setup to measure cellular forces is traction